Data Evaluation Report on the Chronic Toxicity of Rimon to Freshwater Invertebrates - Daphnia sp.
PMRA Submission Number{.....} EPA MRID Number 45638211

Data Requirement:	PMRA DATA CODE	{.....}
	EPA DP Barcode	D285479
	OECD Data Point	
	EPA MRID	45638211
	EPA Guideline	§72-4b

Test material: ^{14}C-"RIMON" **Radiochemical Purity:** >99%
Common name Novaluron
Chemical name: IUPAC: 1,(3-chloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl)-3-(2,6-difluorobenzoyl)urea
CAS name: N-[[[3-Chloro-4-[1,1,2-trifluoro-2-(trifluoromethoxy)ethoxy]phenyl]carbonyl]-2,6-difluorobenzamide
CAS No.: 116714-46-6
Synonyms: None specified

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

QC Reviewer: Christie E. Padova
Staff Scientist, Dynamac Corporation

Signature:
Date: 4/1/03

Primary Reviewer: Bill Evans, Biologist
OPP/EFED/ERB - I

Date: 11/26/03

Reference/Submission No.:

Company Code:
Active Code:
EPA PC Code: 124002

Date Evaluation Completed:

CITATION: Jenkins, C.A. 1998. ^{14}C-"RIMON" - *Daphnia magna* Reproduction Test. Unpublished study performed by Huntingdon Life Sciences Ltd., Eye, Suffolk, England, and sponsored by Makhteshim Chemical Works Ltd., Beer-Sheva, Israel. Laboratory Project ID: MAK/405. Study initiated June 27, 1997 and completed May 12, 1998.

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EXECUTIVE SUMMARY:

The 21-day chronic toxicity of radiolabeled [difluorobenzoyl-(U)^{14}C and chlorophenyl-(U)^{14}C]Rimon (Novaluron) to *Daphnia magna* was studied under static renewal conditions. Nominal concentrations were 0.0 (negative and solvent controls), 6.25, 12.5, 25, 50, and 100 ng/L. Mean-measured concentrations, as determined by LSC, were <LOD (negative and solvent controls), 3.75, 6.90, 14.5, 29.9, and 62.8 ng/L.

No mortalities were observed in the controls, 3.75, or 14.5 ng/L exposure groups. Cumulative mortality was 2.5% in the 6.90 and 29.9 ng/L groups and 57.5% in the 62.8 ng/L group. The NOEC for parental mortality was 29.9 ng/L. The LC_{50} (with 95% C.I.) was 57.9 (50.8-70.7) ng/L. On Day 8, juveniles were present in all of the control vessels and in all of the test vessels at 3.75 to 29.9 ng/L. At the 62.8 ng/L level, juveniles were observed in two of the vessels on Day 8 and in the third vessel on Day 11; no juveniles were produced by the adults in the fourth vessel. The mean cumulative numbers of juveniles produced per adult and the numbers of juveniles produced per adult per vessel were statistically-reduced at the 62.8 ng/L test level. The resultant NOEC for reproduction was 29.9 ng/L. No statistical differences were observed between the mean cumulative numbers of carapaces shed per adult or terminal lengths when compared to pooled controls. The NOEC for parental growth was therefore >62.8 ng/L.

This study is scientifically sound, fulfills the guideline requirments for a chronic toxicity study with freshwater invertebrates [§72-4(b)], and is classified as CORE.

Results Synopsis:

Test Organism Age (eg. 1st instar): <24 hours old
Test Type (Flowthrough, Static, Static Renewal): Static Renewal

Mortality/immobilization:
LC_{50}/EC_{50}: 57.9 ng/L 95% C.I.: 50.8-70.7 ng/L
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of offspring/vessel:
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of offspring/adult/vessel:
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of carapaces:
NOEC: 62.8 ng/L
LOEC: >62.8 ng/L

Length:
NOEC: 62.8 ng/L
LOEC: >62.8 ng/L

Most Sensitive Endpoints: Parental survival and offspring production

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study protocol was based on procedures outlined the OECD Guideline for Testing of Chemicals, Number 202, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, Part 1. Procedure 202 (1984), and U.S. EPA Pesticide Assessment Guidelines, Aquatic Organisms Testing §72-4 Fish Early Life-Stage and Aquatic Invertebrate Life-Cycle (1982). Deviations from U.S. EPA FIFRA guideline §72-4(b) include:

1. The age and pretest health (including mortality) of the parental stock was not specified. In addition, an isolated 21-day acclimation period was not performed.

2. The study design differed from EPA guidance: in this study, 10 daphnids per test chamber were maintained, with four replicate chambers per concentration (total of 40 daphnids/concentration). EPA guidance recommends 22 daphnids/level for static renewal studies, where seven test chambers should contain one daphnid each (to collect data on survival, growth, and reproduction), and three test chambers should contain five daphnids each (to collect data on survival only).

3. A slight overlap of mean-measured concentrations was observed between the 6.25 and 12.5 ng/L groups (nominal).

4. The availability of [^{14}C]Rimon decreased significantly between renewal periods; recoveries of Rimon from "old" solutions were only 48 to 83% of Rimon concentrations in "new" solutions.

5. The water hardness range (47-52 mg/L) was notably less than required (160-180 mg/L).

6. The pH range (7.3-8.0) was slightly less than recommended (7.6-8.0).

7. The particulate matter in the dilution water was not specified.

8. Terminal dry weights were not determined.

Although the availability of Rimon decreased significantly between renewal period, HPLC/RAM results were consistent with LSC results, and it was demonstrated that Rimon accounted for all of the recovered radioactivity during the 21-day study. Since attempts were made prior to study initiation to minimize adsorption of Rimon to the test vessels, the decreases in measured levels were considered unavoidable. Therefore, there were no deviations that affected the validity or acceptability of the study.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. This study was conducted in compliance with the GLP standards of the United Kingdom, EC Council Directive, OECD, U.S. EPA, and Japan Ministry of Agriculture, Forestry and Fisheries (p. 3).

A. MATERIALS:

1. Test Material ^{14}C-"RIMON" (Novaluron)

Description: White solid

Lot No./Batch No. : 3286-018

Purity: >99%

Position of radio-label: Uniformly labeled on both the difluorobenzoyl- and chlorophenyl-rings

Specific activity: 127.1 mCi/mmol

Stability of Compound Under Test Conditions: A 3-day stability trial (longest renewal period) was conducted at 100 ng/L (highest test level). Test medium was sampled on Days 17 ("new") and 20 ("old") and analyzed for ^{14}C-Rimon by HPLC/RAM (p. 17). Results demonstrated that although the availability of Rimon declined significantly (from 98.9 to 62.5 ng/L) between 17 and 20 Days, that Rimon did not degrade significantly in solution, accounting for 93.1-96.0% of the total recovered radioactivity (Table 3, p. 27).

Storage conditions of test chemicals: Frozen (-20°C) and protected from light.

OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound). OECD requirements were not reported.

2. Test organism:

Species: *Daphnia magna*

Age of the parental stock: Not specified

Source: In-house laboratory cultures were maintained since August 1989 (originally from the University of Sheffield, England).

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: A 10-day range-finding study was conducted with ten daphnia/level at nominal concentrations of 0.0032, 0.01, 0.032, 0.1, and 0.32 μg/L (p. 15). The treatment groups were compared to a dilution water control and solvent control (0.1 mL/L). After 10 days, mortality was 10, 30, 10, 20, 20, 30, and 100% in the dilution water control, solvent control, 0.0032, 0.01, 0.032, 0.1, and 0.32 μg/L treatment groups, respectively (Table 1, p. 25). Juveniles were produced in

both control groups and in the 0.1 and 0.32 μg/L test groups only. Results of chemical analysis (LSC) conducted at each test level on Days 0 and 3 (prior to renewal) indicated that the exposure levels were lower than intended (54-70% of nominal) and decreased slightly between renewal of the media (to 37-64% of nominal; p. 18). A 3-day stability experiment was conducted with 0.1 μg/L solution (p. 17). Results (HPLC) indicated that the intended exposure concentration was achieved but decreased slightly during the 72-hour period (to approximately 87% of nominal, p. 18).

b) Definitive Study:

Table 1. Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental acclimation: Period: Conditions (same as test or not): Feeding: Health: (any mortality observed)	 Continuous Same as test Fed at least five times/weekly with a suspension of cultured *Chlorella vulgaris* (2 to 8 x 10^5 cells/mL) and yeast (0.04 mg/L). Not reported	
Test condition: static renewal/flow through: Type of dilution system- for flow through method. Renewal rate for static renewal	 Static renewal N/A 3 times per week	*For flow-through study: consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.*
Aeration, if any	No aeration during the study.	*Dilution water should be aerated to insure DO concentration at or near 100% saturation. Test tanks should not be aerated.*
Duration of the test	21 days	*EPA requires 21 days for static renewal*

Parameter	Details	Remarks *Criteria*
Test vessel Material (glass/stainless steel): Size (for growth and reproduction/survival test): Fill volume:	Glass dishes covered with clear perspex sheets Not specified Approximately 500 mL	To minimize adsorption, all glassware was pre-treated with a siliconizing agent (SurfaSil, 2% in acetone) and also pre-exposed to the test material at the appropriate concentrations for 2 or 3 days prior to use (p. 14). *1. Material: Glass, No. 316 stainless steel, or perfluorocarbon plastics* *2. Size: 250 mL with 200 mL fill volume is preferred; 100 mL with 80 mL fill volume is acceptable.* *OECD requires parent animals be maintained individually, one per vessel, with 50 - 100 mL of medium in each vessel.*
Source of dilution water	The dilution water consisted of treated tap water. Treatment involved blending tap water, previously filtered through activated carbon, with tap water which had been softened and treated by reverse osmosis. In the holding and test areas, this water was further blended with a supply of softened water, treated by reverse osmosis, to reduce the hardness to approximately 50 mg/L as $CaCO_3$ (p. 14).	*Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).*

Parameter	Details	Remarks *Criteria*
Water parameters: Hardness	47-52 as mg of $CaCO_3$/L	The water hardness was less than recommended.
pH	7.3-8.0	The pH range was slightly less than recommended.
Dissolved oxygen	60-104%	Results of the analysis of laboratory diluter water (March and September 1997) for total organic carbon, pesticides, bacteria, and several other parameters were provided in Appendix 2, p. 39.
Temperature	19.9-21.7°C	
Total Organic Carbon	0.8-0.9 mg/L	
Particulate matter	Not specified	
Metals	See Appendix 2, p. 39	*EPA requires:* ***hardness*** *160 to 180 mg/L as $CaCO_3$; OECD requires > 140 mg/L as $CaCO_3$* ***pH*** *7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours. OECD requires pH rang 6 - 9 and should not vary more than 1.5 units in any one test.* ***Dissolved Oxygen*** *Renewal: must not drop below 50% for more than 48 hours. Flow-through: ≥ 60% throughouttest.* ***Temperature*** *20 °C ± 2 °C. Must not deviate from 20 °C by more than 5 °C for more than 48 hours. OECD requires range 18 - 22°C; temperature should not vary more than ± 2°C* *OECD requires total organic carbon < 2 mg/L*
Pesticides	Not detected	
Chlorine	Not detected	

Parameter	Details	Remarks *Criteria*
Number of organisms: For growth and reproduction: For survival test:	40 daphnids/test level 4 chambers with 10 daphnids per chamber (Not differentiated; same test chambers as above)	Did not follow recommended test design. *EPA requires 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.* *OECD requires minimum of 10 daphnids held individually for static tests. For flow-through tests, 40 animals divided into 4 groups of 10 animals at each test concentration.*
Application rates nominal: measured:	0.0 (negative and solvent controls), 6.25, 12.5, 25, 50, and 100 ng/L <LOD (negative and solvent controls), 3.75, 6.90, 14.5, 29.9, and 62.8 ng/L	Mean-measured concentrations were based on LSC analysis of "new" and "old" solutions at each renewal period (Table 2, p. 26). Recoveries were consistently low and highly variable, with high/low ratios exceeding 1.5 at all test levels. Only at the 25 ng/L (nominal) level were measured concentrations of "old" solutions ≥ 70% of "new" solutions. A slight overlap of mean-measured concentrations was observed between the 6.25 and 12.5 ng/L groups (nominal). *EPA requires control(s) and at least 5 test concentrations; dilution factor not greater than 50%.* *OECD requires at least 5 test concentrations in a geometric series with a separation factor not exceeding 3.2.*
Solvent (type, percentage, if used)	Acetone, 0.1 mL/L	*EPA requires:* *solvent to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests. Acceptable solvents are dimethylformamide, triethylene glycol, methanol, acetone and ethanol.* *OECD requires ≤ 0.1 mL/L*

Parameter	Details	Remarks *Criteria*
Lighting	16:8 hour light/dark cycle with a transition period	The light intensity was approximately 476 lux (p. 20). *EPA/OECD requires: 16 hours light, 8 hours dark.*
Stability of chemical in the test system	A 3-day stability trial (longest renewal period) was conducted at 100 ng/L (highest test level). Test medium was sampled on Days 17 ("new") and 20 ("old") and analyzed for ^{14}C-Rimon by HPLC/RAM (p. 17). Results demonstrated that although the availability of Rimon declined significantly (from 98.9 to 62.5 ng/L) between 17 and 20 Days, that Rimon did not degrade significantly in solution, accounting for 93.1-96.0% of the total recovered radioactivity (Table 3, p. 27).	Based on LSC analysis of "new" test medium sampled on Days 0, 10, and 17 and "old" test medium sampled on Days 3, 13, and 19, the availability of radioactivity decreased significantly between renewal periods. Recoveries were 64-85% of nominal concentrations for "new" test medium and 32-70% of nominal concentrations for "old" test medium. Only at the 25 ng/L (nominal) level were measured concentrations of "old" solutions ≥ 70% of "new" solutions.
Recovery of chemical: Frequency of measurement: LOD: LOQ:	64-85% of nominal for "new" test medium 32-70% of nominal for "old" test medium Samples analyzed on Days 0 (new), 3 (old), 10 (new), 13 (old), 17 (new), and 19 (old). 0.005 ng/mL (p. 40) Not reported	Recoveries are for total radioactive residues, as determined by LSC analysis (Table 2, p. 26). Radioactivity declined significantly between the "new" and "old" solutions.
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks *Criteria*
Data end points measured (list)	- Survival/immobility of first-generation daphnids - Number of carapaces shed per adult - Day of first juvenile release - Number of young produced per adult - Terminal length	In addition to length, terminal dry weights should have been measured. *EPA requires:* *- Survival of first-generation daphnids,* *- Number of young produced per female,* *- Dry weight (recommended) and length (required)* of each first generation daphnid alive at the end of the test,* *- Observations of other effects or clinical signs.* **current requirement until the Agency provides specific guidance indicating otherwise (Pesticide Rejection Rate Analysis, p. 132).*
Observation intervals	Mortality/immobility and number of carapaces shed were recorded daily. The numbers of juveniles present were determined at media renewal (three times/week). Total length was determined at the end of the test (Day 21).	
Were raw data included?	Yes, adequate.	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

No mortalities were observed in the controls, 3.75, or 14.5 ng/L (mean-measured) exposure groups (Table 4, p. 28). Cumulative mortality was 2.5% in the 6.90 and 29.9 ng/L groups and 57.5% in the 62.8 ng/L group. The NOEC for parental mortality was 29.9 ng/L.

Table 1: Effect of Rimon on growth and survival of Daphnia sp.

Treatment (ng/L) Measured (and Nominal) Concentrations	Mortality (dead or immobile)		Mean No. Young per Adult	Mean Number of Carapaces Shed per Adult	Length (mm)	Day of 1st Brood
	No Dead	%				
Negative control	0	0	90.2	6.7	3.81	8
Solvent control	0	0	102	6.8	3.90	8
3.75 (6.25)	0	0	97.9	6.4	3.96	8
6.90 (12.5)	1	2.5	102	6.9	3.99	8
14.5 (25)	0	0	107	6.8	3.93	8
29.9 (50)	1	2.5	107	6.9	3.93	8
62.8 (100)	23	57.5	8.08*	6.2	3.37**	8 and 11
NOEC, ng/L	29.9		29.9	62.8	29.9	29.9
LOEC, ng/L	62.8		62.8	>62.8	62.8	62.8
MATC, ng/L	ND		ND	ND	ND	ND
LC_{50}/EC_{50} (95% C.I.), ng/L	57.9 (50.8-70.7)		46.7 (29.9-62.8)	ND	ND	ND

*Values with significant differences versus solvent control ($p<0.05$), as determined by the study authors.
**Values with significant differences versus solvent control ($p<0.001$), as determined by the study authors.
ND = Not determined.

B. EFFECT ON REPRODUCTION AND GROWTH:

On Day 8, juveniles were present in all of the control vessels and in all of the test vessels at 3.75 to 29.9 ng/L. At the 62.8 ng/L level, juveniles were observed in two of the vessels on Day 8 and in the third vessel on Day 11; no juveniles were produced by the adults in the fourth vessel. The mean cumulative numbers of juveniles produced per adult was statistically-reduced at the 62.8 ng/L test level. The resultant NOEC for reproduction was 29.9 ng/L.

The mean cumulative numbers of carapaces shed per adult was unaffected by treatment with Rimon. Terminal length, however, was statistically-reduced at the 62.8 ng/L level. The NOEC for parental growth was 29.9 ng/L.

C. REPORTED STATISTICS:

The parental LC_{50} (with 95% C.I.) was calculated using the moving average method. The number of dead parental daphnia, the mean number of carapaces shed per adult, the total number of juveniles, and the mean number of juveniles produced per adult in the solvent control group were compared with those in the test

groups by Dunnett's multi-comparison test (95% level of probability). The reproduction EC_{50} (with 95% C.I.) was calculated using the binomial test. Length data were compared to those in the solvent control group using the Kruskal-Wallis variance analysis test and Wilcoxon Rank sum test. Mean-measured values, as determined by LSC, were used in all estimations.

D. VERIFICATION OF STATISTICAL RESULTS:

NOEC and LOEC for parental survival were visually determined. The LC_{50} was determined using the moving average angle method via TOXANAL statistical software. Since no differences were detected between the solvent and negative control groups, these groups were pooled for subsequent analyses. Data for the number of juveniles per vessel and the number of juveniles per adult per vessel were confirmed to be normally distributed and the variances were homogeneous; the NOEC and LOEC for these endpoints were determined using ANOVA followed by William's test via TOXSTAT statistical software. The number of carapaces was analyzed using ANOVA. Terminal length data were normally distributed, but the variances were not homogeneous. The NOEC and LOEC for this endpoint were determined using the non-parametric Wilcoxon Rank Sum test with Bonferonni adjustment and the Kruskal Wallis test via TOXSTAT statistical software. Mean-measured values, as determined by LSC, were used in all estimations.

Mortality/immobilization:
LC_{50}/EC_{50}: 57.9 ng/L 95% C.I.: 50.8-70.7 ng/L
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of offspring/vessel:
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of offspring/adult/vessel:
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of carapaces:
NOEC: 62.8 ng/L
LOEC: >62.8 ng/L

Length:
NOEC: 62.8 ng/L
LOEC: >62.8 ng/L

Most Sensitive Endpoints: Parental survival and offspring production

E. STUDY DEFICIENCIES:

Based on LSC analyses, concentrations of available radioactivity declined significantly between each renewal period: recoveries ranged from 64-85% of nominal for "new" test medium and 32-70% of nominal for "old" test medium (Table 2, p. 26). The high/low ratio exceeded 1.5 for all test levels (reviewer determined), and only at the 25 ng/L (nominal) level were measured concentrations of "old" solutions $\geq$ 70% of "new" solutions. However, based on the HPLC/RAM analysis of the highest test level, it was observed that [^{14}C]Rimon did not

undergo chemical degradation and accounted for 93.1-96.0% of the recovered radioactivity over a 3-day period (the highest renewal interval; Table 3, p. 27). Low and declining recoveries were also observed in a 10-day preliminary experiment: results of chemical analysis (LSC) conducted at each test level on Days 0 and 3 (prior to renewal) indicated that the exposure levels were lower than intended (54-70% of nominal) and decreased slightly between renewal of the media (to 37-64% of nominal; p. 18). A 3-day stability experiment was conducted with 0.1 μg/L solution (p. 17). Results (HPLC) indicated that the intended exposure concentration was achieved but decreased slightly during the 72-hour period (to approximately 87% of nominal, p. 18). In attempts to minimize adsorption, all glassware was pre-treated with a siliconizing agent (SurfaSil, 2% in acetone) and also pre-exposed to the test material at the appropriate concentrations for 2 or 3 days prior to use (p. 14). The study author reported that the losses observed in the definitive experiment may have been due to adsorption onto or uptake by the *Daphnia* and algal cells although there was no clear trend in the data to support this, and that adsorption onto the glassware used was considered unlikely because of the pre-treatment steps taken (p. 18). The study author also reported that the decrease in measured levels were unavoidable and is not thought to have affected the integrity of the test (p. 22). Since the decline of the test material was adequately addressed by the study author, this decline would alone not affect the acceptability of this study (refer to the Pesticide Reregistration Rejection Rate Analysis, Ecological Effects, Appendix A: Additional Supporting Documents, Conducting Acceptable Aquatic Lab Studies: Proposed Guidance, pp. 3-9). This study satisfies guideline requirements and is classified as CORE.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were nearly identical to the study author's. The study author's NOEC and LOEC estimates for terminal length were lower than the reviewer's because the study author compared the treatment groups to the solvent control group only. The reviewer's results are reported in the Executive Summary and Conclusions sections of this DER.

To minimize adsorption, all glassware was pre-treated with a siliconizing agent (SurfaSil, 2% in acetone) and also pre-exposed to the test material at the appropriate concentrations for 2 or 3 days prior to use (p. 14). The study author reported that radioactive losses may have been due to adsorption onto or uptake by the *Daphnia* and algal cells although there was no clear trend in the data to support this (p. 18). It was also reported that adsorption onto the glassware used during the test was considered unlikely because of the pre-treatment measures.

During HPLC/RAM analyses, samples were analyzed for parent Rimon, and also for the potential metabolites 2,6-difluorobenzoic acid; 2,6-difluorobenzamide; and 4-amino-2-chlorophenol (p. 41).

At all concentrations, the test media were clear and colorless on the days of preparation (p. 20).

G. CONCLUSIONS:

This study is scientifically sound, fulfills U.S. EPA guideline §72-4(b), and is classified as CORE. The most sensitive endpoints were parental survival and offspring production.

Mortality/immobilization:
LC_{50}/EC_{50}: 57.9 ng/L 95% C.I.: 50.8-70.7 ng/L
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of offspring/vessel:
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of offspring/adult/vessel:
NOEC: 29.9 ng/L
LOEC: 62.8 ng/L

Number of carapaces:
NOEC: 62.8 ng/L
LOEC: >62.8 ng/L

Length:
NOEC: 62.8 ng/L
LOEC: >62.8 ng/L

Most Sensitive Endpoints: Parental survival and offspring production

III. REFERENCES:

OECD Guidelines for Testing of Chemicals. "*Daphnia*, sp., Acute Immobilisation Test and Reproduction Test, Part 1. Procedure 202, adopted 4 April 1984.

US EPA Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms, Series 72, Aquatic Organisms Testing §72-4 Fish early life-stage and aquatic invertebrate life-cycle, October 1982.

Stephan, C.E. 1977. Methods for Calculating an LC50. Aquatic Toxicology and Hazard Evaluation. ASTM STP 634.

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Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. Journal of the American Statistical Association 50, 1096-1121.

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Kruskal, W.H. and Wallis, W.A. 1952. "Use of Ranks in One-Criterion Variance Analysis". Journal of the American Statistical Association 47:583-621 (Kruskal-Wallis test).

Wilcoxon, F. 1945. "Individual comparisons by ranking methods". Biometrics Bulletin 1:80-83 (Wilcoxon Rank sum test).

Huntingdon Life Sciences Report number MAK404/970470. "RIMON" Technical: Acute Toxicity to *Daphnia magna*.

APPENDIX 1. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

LC50

Moving Average Angle Method

	LC50	95 % Confidence Limit		
1	.1059372	**57.88646**	**50.76392**	**70.69715**

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	8.388705	19.7458	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.429246
95 PERCENT CONFIDENCE LIMITS =-6.502968 AND 13.36146

LC50 = 64.14183
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 27.33992
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

NOEC and LOEC

Length

length
File: 82111 Transform: NO TRANSFORMATION

WILCOXON RANK SUM TEST W/ BONFERRONI ADJUSTMENT - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	REPS	SIG
1	GRPS 1&2 POOLED	3.757				
2	3.75	3.953	38.50	12.00	4	
3	6.90	3.995	42.00	12.00	4	
4	14.5	3.935	38.50	12.00	4	
5	29.9	3.933	38.00	12.00	4	
6	62.8	3.395	14.00	12.00	4	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

length
File: 82111 Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	GRPS 1&2 POOLED	3.757	3.757	75.000
2	3.75	3.953	3.953	79.500
3	6.90	3.995	3.995	98.500
4	14.5	3.935	3.935	71.000
5	29.9	3.933	3.933	68.000
6	62.8	3.395	3.395	14.000

Calculated H Value = 19.067 Critical H Value Table = 11.070
Since Calc H > Crit H REJECT Ho:All groups are equal.

length
File: 8211l Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 6	0 1	0 5	0 4	0 2	0 3
6	62.8	3.395	3.395	\					
1	GRPS 1&2 POOLED	3.757	3.757	.	\				
5	29.9	3.933	3.933	.	.	\			
4	14.5	3.935	3.935	.	.	.	\		
2	3.75	3.953	3.953	.	.	.	.	\	
3	6.90	3.995	3.995	*	*	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,6) = 2.936 Unequal reps - multiple SE values

 # carapaces
mean number of carapaces shed/adult
File: 8211c Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.546	0.309	2.512
Within (Error)	22	2.702	0.123	
Total	27	4.248		

Critical F value = 2.66 (0.05,5,22)
Since F < Critical F **FAIL TO REJECT Ho:All groups equal**

mean number of carapaces shed/adult
File: 8211c Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	6.738	6.738		
2	3.75	6.375	6.375	1.688	
3	6.9	6.890	6.890	-0.710	
4	14.5	6.800	6.800	-0.291	
5	29.9	6.855	6.855	-0.547	
6	62.8	6.233	6.233	2.351	

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

mean number of carapaces shed/adult
File: 8211c Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	3.75	4	0.539	8.0	0.363
3	6.9	4	0.539	8.0	-0.152
4	14.5	4	0.539	8.0	-0.063
5	29.9	4	0.539	8.0	-0.118
6	62.8	4	0.539	8.0	0.505

mean number of carapaces shed/adult
File: 8211c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	6.738	6.738	6.738
2	3.75	4	6.375	6.375	6.730
3	6.9	4	6.890	6.890	6.730
4	14.5	4	6.800	6.800	6.730
5	29.9	4	6.855	6.855	6.730
6	62.8	4	6.233	6.233	6.233

mean number of carapaces shed/adult
File: 8211c Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	6.738				
3.75	6.730	0.035		1.72	k= 1, v=22
6.9	6.730	0.035		1.80	k= 2, v=22
14.5	6.730	0.035		1.83	k= 3, v=22
29.9	6.730	0.035		1.84	k= 4, v=22
62.8	6.233	2.353	*	1.85	k= 5, v=22

s = 0.350
Note: df used for table values are approximate when v > 20.

 # Juveniles/vessel
juveniles
File: 8211j Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	3307132.304	661426.461	106.826
Within (Error)	22	136216.375	6191.653	
Total	27	3443348.679		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F REJECT Ho:All groups equal

juveniles
File: 8211j Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	963.375	963.375		
2	3.75	981.000	981.000	-0.366	
3	6.90	1000.500	1000.500	-0.770	
4	14.5	1069.250	1069.250	-2.197	
5	29.9	1037.000	1037.000	-1.528	
6	62.8	25.750	25.750	19.459	*

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

juveniles
File: 8211j Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	3.75	4	120.898	12.5	-17.625
3	6.90	4	120.898	12.5	-37.125
4	14.5	4	120.898	12.5	-105.875
5	29.9	4	120.898	12.5	-73.625
6	62.8	4	120.898	12.5	937.625

juveniles
File: 8211j Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	963.375	963.375	1002.417
2	3.75	4	981.000	981.000	1002.417
3	6.90	4	1000.500	1000.500	1002.417
4	14.5	4	1069.250	1069.250	1002.417
5	29.9	4	1037.000	1037.000	1002.417
6	62.8	4	25.750	25.750	25.750

juveniles
File: 8211j Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	1002.417				

3.75	1002.417	0.810		1.72	k= 1, v=22
6.90	1002.417	0.810		1.80	k= 2, v=22
14.5	1002.417	0.810		1.83	k= 3, v=22
29.9	**1002.417**	**0.810**		**1.84**	**k= 4, v=22**
62.8	25.750	19.459	*	1.85	k= 5, v=22

s = 78.687
Note: df used for table values are approximate when v > 20.

 # Juveniles/adult/vessel
juveniles/adult/vessel
File: 8211ja Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	30113.251	6022.650	48.833
Within (Error)	22	2713.303	123.332	
Total	27	32826.553		

Critical F value = 2.66 (0.05,5,22)
Since F > Critical F REJECT Ho:All groups equal

juveniles/adult/vessel
File: 8211ja Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	96.250	96.250		
2	3.75	97.925	97.925	-0.246	
3	6.90	101.800	101.800	-0.816	
4	14.5	107.000	107.000	-1.581	
5	29.9	106.875	106.875	-1.562	
6	62.8	8.085	8.085	12.964	*

Bonferroni T table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

juveniles/adult/vessel
File: 8211ja Transform: NO TRANSFORMATION

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	3.75	4	17.063	17.7	-1.675
3	6.90	4	17.063	17.7	-5.550
4	14.5	4	17.063	17.7	-10.750
5	29.9	4	17.063	17.7	-10.625
6	62.8	4	17.063	17.7	88.165

juveniles/adult/vessel

File: 8211ja Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	96.250	96.250	101.017
2	3.75	4	97.925	97.925	101.017
3	6.90	4	101.800	101.800	101.017
4	14.5	4	107.000	107.000	101.017
5	29.9	4	106.875	106.875	101.017
6	62.8	4	8.085	8.085	8.085

juveniles/adult/vessel
File: 8211ja Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	101.017				
3.75	101.017	0.701		1.72	k= 1, v=22
6.90	101.017	0.701		1.80	k= 2, v=22
14.5	101.017	0.701		1.83	k= 3, v=22
29.9	**101.017**	**0.701**		**1.84**	**k= 4, v=22**
62.8	8.085	12.964	*	1.85	k= 5, v=22

s = 11.105
Note: df used for table values are approximate when v > 20.